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Biosurfactant-biopolymer driven microbial enhanced oil recovery (MEOR) and its optimization by an ANN-GA hybrid technique

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ABSTRACT

A lipopeptide biosurfactant produced by marine *Bacillus megaterium* and a biopolymer produced by thermophilic *Bacillus licheniformis* were tested for their application potential in the enhanced oil recovery. The crude biosurfactant obtained after acid precipitation effectively reduced the surface tension of deionized water from 70.5 to 28.25 mN/m and the interfacial tension between lube oil and water from 18.6 to 1.5 mN/m at a concentration of 250 mg L^{-1}. The biosurfactant exhibited a maximum emulsification activity (E_{24}) of 81.66% against lube oil. The lipopeptide micelles were stabilized by addition of Ca^{2+} ions to the biosurfactant solution. The oil recovery efficiency of Ca^{2+} conditioned lipopeptide solution from a sand-packed column was optimized by using artificial neural network (ANN) modelling coupled with genetic algorithm (GA) optimization. Three important parameters namely lipopeptide concentration, Ca^{2+} concentration and solution pH were considered for optimization studies. In order to further improve the recovery efficiency, a water soluble biopolymer produced by *Bacillus licheniformis* was used as a flooding agent after biosurfactant incubation. Upon ANN-GA optimization, 45% tertiary oil recovery was achieved, when biopolymer at a concentration of 3 g L^{-1} was used as a flooding agent. Oil recovery was only 29% at optimal conditions predicted by ANN-GA, when only water was used as flooding solution. The important characteristics of biopolymers such as its viscosity, pore plugging capabilities and bio-cementing ability have also been tested. Thus, as a result of biosurfactant incubation and biopolymer flooding under the optimal process conditions, a maximum oil recovery of 45% was achieved. Therefore, this study is novel, timely and interesting for it showed the combined influence of biosurfactant and biopolymer on solubilisation and mobilization of oil from the soil.

1. Introduction

Microbial enhanced oil recovery (MEOR) is a tertiary oil recovery technique that employs microbes and their by- products to mobilize oil in the reservoir. In MEOR, the injection of microbes and their by-products cause some beneficial effects such as formation of stable emulsions, mobilization of oil as a result of reduction in interfacial tension between oil and rocks surfaces, and improving the flow of injection fluid, which help push the residual oil effectively by clogging the so-called thief-zones (Patel et al., 2015; Sen, 2008). Microbial metabolites such as biosurfactants, biopolymers, acids, gases, solvents and enzymes have played significant roles in improving the sweep efficiency of oil (Patel et al., 2015; Sen, 2008). Although, the overall efficiency of MEOR is decided by a number of factors including that of reservoir properties such as lithology, nature of the sands, porosity, permeability, reservoir temperature, crude oil gravity and the drive types, the important factor which the users have better control over and thus need greater attention is workability of injection and flooding fluids. By all means, the primary goal of MEOR program is to further improve the oil recovery by designing a better injection liquid, which can facilitate a better reduction in the interfacial tension between oil and solid surface of rocks, where the oil is tightly held by capillary forces.

Bacterially derived biosurfactants for MEOR applications are selected based on their sustenance to withstand adverse reservoir conditions such as high temperature, salinity and pressure deep inside the reservoir (Mukherjee et al., 2006; Youssef et al., 2007). So far microbially synthesized biosurfactants such as rhamnolipids and lipo-

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peptides have been widely reported for use in enhanced oil recovery (Amani, 2015; Gudina et al., 2012; Liu et al., 2015). Despite improved characteristics of lipopeptide over other biosurfactants, their use in MEOR until recently has been the subject of debate, because of its low yield and high production cost. However, their improved yield and bio-manufacturing economics in recent years, thanks to the use of cheaper substrates (Dhanarajan et al., 2014; Dhanarajan et al., 2015) and adoption of various process intensification strategies (Rangarajan and Clarke, 2015; Rangarajan et al., 2012; Rangarajan et al., 2015; Winterburn et al., 2011), have removed speculations about its use for MEOR applications. Also, only a fewer efforts have been made to modify the characteristics of lipopeptides that can improve the work-ability of lipopeptides under adverse conditions of MEOR. To this end, of a few strategies reported; the one using divalent cations to enhance the surface activity seem to be very promising as it formed stable micelles and vesicles by manipulating their aggregation abilities (Rangarajan et al., 2014). It is strongly believed that the Ca^{2+} conditioned lipopeptide with enhanced characteristics would result in enhanced oil recovery.

Another important aspect in MEOR is oil-sweeping efficiency by flooding solution, where the choice of flooding solution plays a crucial role in determining the sweeping efficiency of the resulting emulsion or oil. Water, by itself, has poor sweep efficiency because of its low viscosity. Thus to increase the viscosity, synthetic polymers such as polyacrylamide and partially hydrolyzed polyacrylamide, modified natural polymers like hydroxyl ethyl cellulose, guar gum, sodium carboxy methyl cellulose and microbially derived polymer like xanthan gum have been used (Abidin et al., 2012; Chen et al., 2013; Sandvik and Maerker, 1977). An important aspect of the polymer is its thermal stability at the persisting high- well temperature. Biopolymers can become a viable alternative to chemically synthesized polymer only if their thermal compatibility is proven to be close to or better than that of synthetic polymer. In addition to being used as flooding agents, polymers can also be used as selective plugging agents to seal the thief zones, whose existence would otherwise reduce the sweeping efficiency of flooding solutions by allowing the preferential flow of injection solution through low pressure zones. However, in order to have a successful pore- plugging, *in situ* production of polymers by thermophiles is usually encouraged, before the oil recovery process. Thus, a biopolymer with desired characteristics has to be identified and tested in lab to evaluate it applicability as a flooding or pore plugging agent.

The objectives of the current work were to assess the application potential crude lipopeptide and crude biopolymer of bacterial origin for EOR. Furthermore, an attempt was made to improve the performance of lipopeptide-based injection solution for EOR from a sand-packed column, by optimizing lipopeptide concentration, pH and Ca^{2+} ions concentration of injection solution using ANN coupled with GA. Biopolymer synthesized by *Bacillus licheniformis* was used as a viscosifier in the flooding water.

2. Materials and methods

2.1. Chemicals

All the chemicals used in the current work were of analytical reagent grade purchased from Merck, India.

2.2. Production of EOR additive agents

2.2.1. Lipopeptide production

Lipopeptide was produced using marine *Bacillus megaterium* (isolated from Andaman and Nicobar Islands, India) using noodle processed water supplemented with chemical fertilizers as described previously (Dhanarajan et al., 2014). After 48 h of fermentation, the culture broth was centrifuged at 10000 rpm for 10 min to separate the cells. The cell free supernatant was acidified to pH 2.0 using 6N HCl to precipitate the biosurfactant. The precipitate was centrifuged and the pellet was lyophilized to get the biosurfactant in crude form. The biosurfactant product was found to contain a mixture of iturin (23%), fengycin (44%) and surfactin (33%) type of lipopeptides (Dhanarajan et al., 2016).

Table 1
Levels chosen for the Central Composite Design.

Level	Biosurfactant Concentration (mg/l)	Ca^{2+} Concentraion (mg/l)	pH
(−1)	260	22	7.2
(+1)	1000	80	10

Table 2
Percentage enhanced oil recovery for 20 sand pack column runs from CCD.

S. No	Biosurfactant Concentration (mg/l)	Ca^{2+} Concentration (mg/l)	pH	%Recovery[a]
1	260	22	7.2	26
2	1000	22	7.2	31
3	260	80	7.2	20
4	1000	80	7.2	41
5	260	22	10	24
6	1000	22	10	30
7	260	80	10	19
8	1000	80	10	33
9	7.74	51	8.6	14
10	1252.26	51	8.6	40
11	630	2.23	8.6	22
12	630	99.97	8.6	28.5
13	630	51	6.25	16
14	630	51	10.95	21
15	630	51	8.6	26
16	630	51	8.6	28
17	630	51	8.6	29
18	630	51	8.6	27
19	630	51	8.6	28
20	630	51	8.6	29

[a] Values are expressed as average of two experiments and percentage deviation is within 2%.

Table 3
Effect of lipopeptide concentrations on emulsification index with crude oil.

Concentration (mg/l)	Emusification index (Mean ± SD)
25	–
50	–
100	3.55
250	26.33 ± 0.33
500	35 ± 1.67
1000	66.66 ± 3.33
2500	81.66 ± 3.33

2.2.2. Biopolymer production

Biopolymer was produced using a thermophilic *Bacillus licheniformis* isolated from hot springs, Agni Kund at Bakreshwar (23° 52′ 48″ N and 87° 22′ 12″ E) in Birbhum district of West Bengal, India. The surface temperature of the hot springs was found to be in the range of 80 °C–90 °C. The organism was grown in Glucose Mineral Salt Medium (GMSM). The composition of the GMSM (Rangarajan et al., 2012) were as follows: Glucose – 25 g L^{-1}, NH_4NO_3–6 g L^{-1}, KH_2PO_4–0.028 g L^{-1}, K_2HPO_4–1.6 g L^{-1}, $MgSO_4$–0.3 g L^{-1} and $CaCl_2{\cdot}2H_2O$ – 0.2 g L^{-1}, $FeSO_4{\cdot}7H_2O$ – 0.1 g L^{-1}. After 24 h of incubation, cells were harvested at 7500 rpm for 10 min. A highly viscous supernatant liquid was obtained. The extracellular product, biopolymer was precipitated from the viscous supernatant by using 2.5 vols of methanol. The precipitate was obtained by centrifuging the liquid, which was then lyophilised to get a white powdered product.

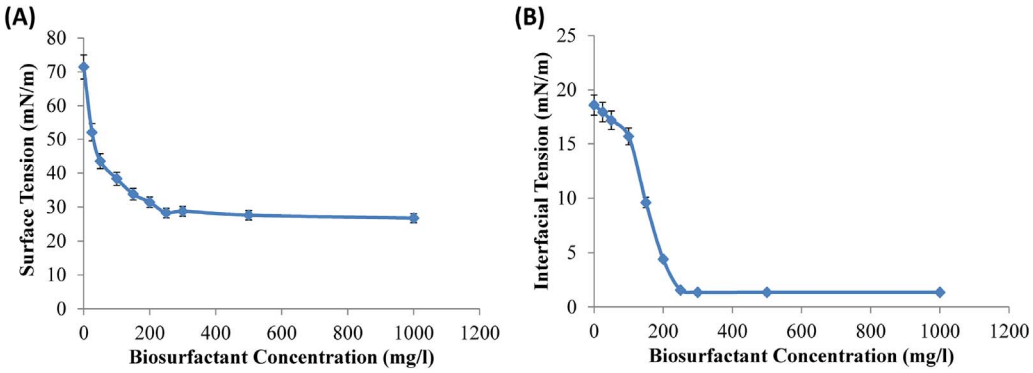

Fig. 1. (a) Variation of surface tension with concentration of biosurfactant. (b) Variation of Interfacial Tension with concentration of Biosurfactant.

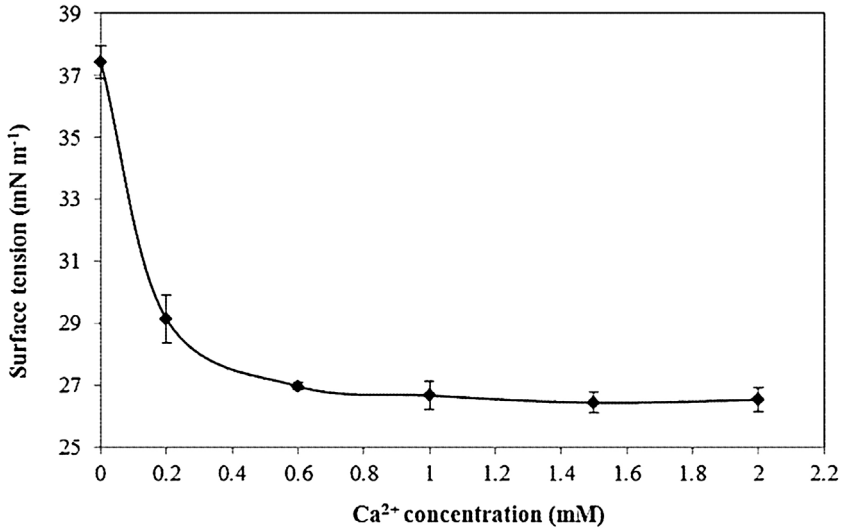

Fig. 2. Effect of Ca^{2+} on the surface tension of lipopeptide solution (lipopeptide concentration was = 50 mg L^{-1}).

2.3. Assessment of EOR application potential of biosurfactant

2.3.1. Emulsification activity measurement

Emulsification activity of the crude lipopeptide (25–2500 mg mL^{-1}) was measured by adding an equal volume of biosurfactant solution and lube oil and mixing at a high speed for 10 min. The height of the emulsion layer was measured after 24 h. Emulsification index (E_{24}%) was estimated as the percentage of height of the emulsion layer upon the total mixture height.

2.3.2. Effect of change in lipopeptide concentrations on surface tension and interfacial tension between lube oil and water

Biosurfactant solutions of varying lipopeptide concentrations 25–200 mg L^{-1} were prepared. The pH of all samples was adjusted to 8 before measurement. The surface and interfacial tension of the solutions was determined using tensiometer by Du Noüy ring method using a tensiometer (Lauda TD 1C, Germany).

2.3.3. Effect of Ca^{2+} ions on surface tension of lipopeptide solutions

Supplementation of varying concentrations (0–2 mM) of Ca^{2+} on surface tension of lipopeptide was evaluated by adding appropriate volume Ca^{2+} ions prepared as $CaCl_2{\cdot}2H_2O$ stock solution. The pH of the sample solutions were maintained at 8.

2.4. Assessment of EOR application potential of biopolymer

2.4.1. FTIR characterization of biopolymer

FTIR analysis of partially purified biopolymer was performed using a Nexus-890 Fourier transform infrared spectrophotometer (Thermo Electron co., Yokohama, Japan). The matrix used was Spectral grade KBr (Merck, Darmstadt, Germany). About 25 mg of sample was uniformly dispersed in KBr powder and subsequently made into a pellet by applying 5–6 ton cm^{-2} of pressure for about 10 min using the hydraulic press (Specac, UK) instrument. Scanning of the sample was carried out in the transmittance mode over the range of 400–4000 cm^{-1}

2.4.2. Rheological studies

The viscosity of product at different concentrations in water was measured at 30 °C, using rheometer (Model: Physica MCR 301; Anton paar, Graz, Austria) with 25 mm parallel plate geometry.

2.4.3. Bio-cementing studies

The biopolymer for its impending bio-cementing application was evaluated by performing various experiments with fine sand. The powdered product was mixed with sand in appropriate ratio (1:20 and 1:10% w/w) and water was added to the mixture. The semisolid mixture was cast into a cylindrical solid with dimensions of 25 mm diameter and 21 mm thickness. The cylindrical block was dried overnight in hot air oven at 50 °C, which weighed 22.3 g. The mechanical strength of the solid sand block for its compression strength was measured using Universal Testing Machine; Housefield-H25KS System (Instron, Norwood, MA, USA) equipped with a Model 2519-104 force transducer. The solid block was compressed between two parallel plates using a load cell with maximum loading of 5000 N and with a compression rate of 1 mm per min as per ASTM D695 – compression inbuilt testing protocol.

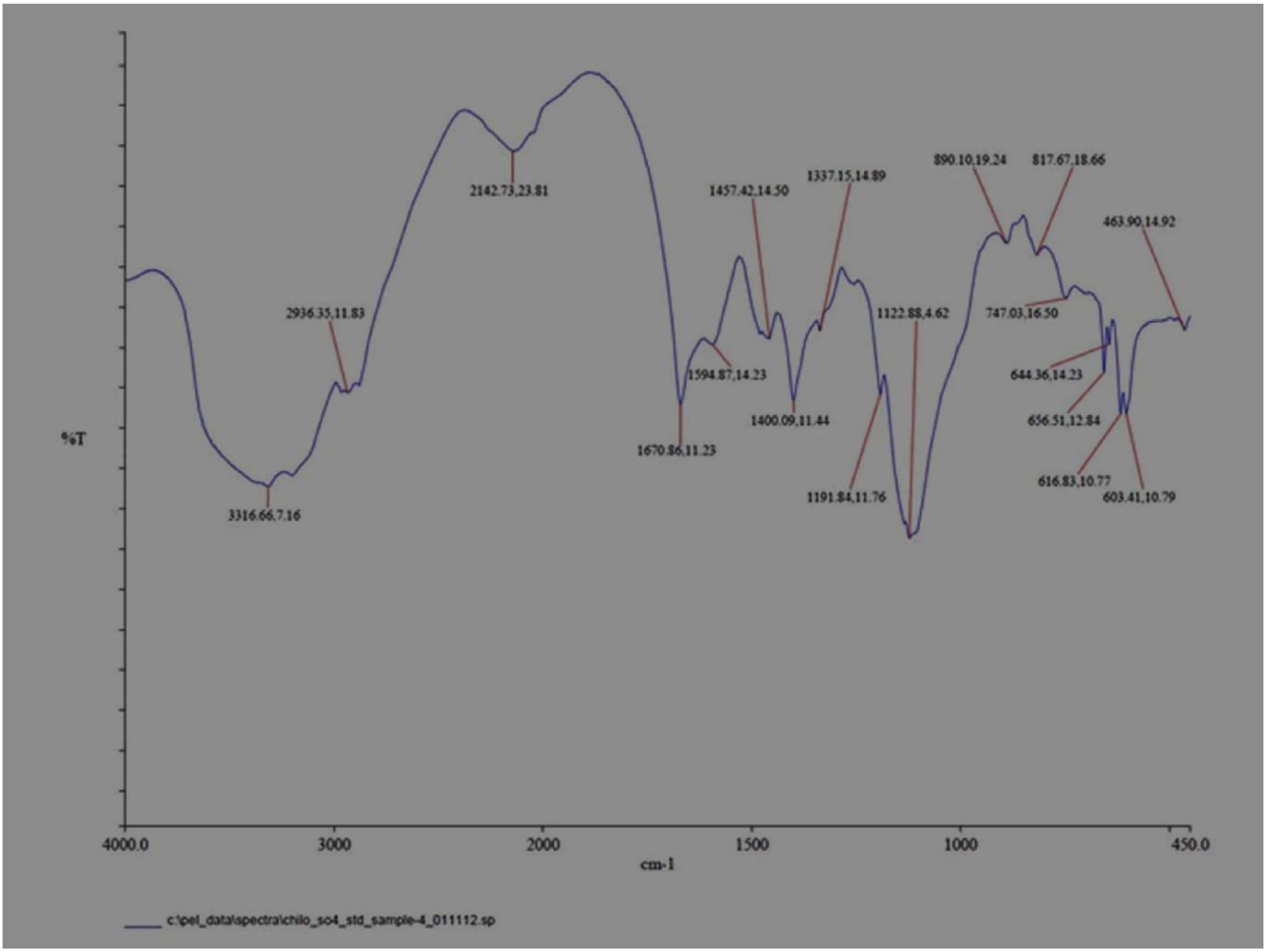

Fig. 3. FTIR spectrum of biopolymer.

Table 4
Peak positions of biopolymer as observed in FTIR spectrum.

Peak position cm^{-1}	Peak assignment
1400.09	δ (CH), δ (OH)
1337.15	δ (CH), δ (OH)
1267.2	ν (CH)
1191.84	ν(CO) linkage
890.10	ν(CO) furanose link
817.67	ν(CO) furanose link

2.4.4. Bio-plugging studies

The potential of the product for its application in EOR was evaluated by studying its bio-plugging ability. The capability of the polymer to selectively plug the pores deep inside the oil well has been reported as one of the mechanisms to enhance the oil recovery (Sen, 2008). Sand columns were used in the current study to evaluate the pore plugging ability of the product. For bioplugging studies, sand was thoroughly mixed with suitable proportion of product in the presence of known quantity of water. The slurry mixture obtained was packed into syringe columns and was allowed to dry for a day at 50 °C. Finally a layer of dry sand is placed over the packed column.

Bio-plugging ability was evaluated by adding a known volume of water over the sand packed column and the percolation of water through sand by wetting action was observed over time. The bio-plugging efficiency was expressed in terms of water dripping rate. Column packed only with sand served as the control.

2.5. Flooding studies in sand-packed column using bioproducts

Oil recovery studies were performed using a 4 cm dia × 10 cm height column packed with sand of ~150 µm particle size. The pore volume of the bed was calculated by measuring the volume of brine required to saturate the column. Then lube oil (density 0.918 kg L^{-1}) was passed into the column until it was completely saturated with oil. After 24 h of incubation, the sand packed column was again flooded with three pore volumes of brine and the amount of oil recovered during this process was noted. The volume of residual oil present in the column after brine flooding was estimated by measuring the difference between the initial volume required to saturate the column and the volume recovered with brine flooding. The oil remaining in the column was recovered by treating with lipopeptide solution at different pH values and ionic strength. Parameters such as lipopeptide concentration, pH and Ca^{2+} ions concentrations in the injection solution was optimized to improve the oil recovery efficiency as given in the following section. The final flooding solution contained 3 g L^{-1} of crude biopolymer.

2.6. Optimization of process variables using ANN-GA for enhanced oil recovery

2.6.1. Experimental design

Based on our preliminary experiments, the range and levels of the three independent parameters namely lipopeptide concentration, pH of the solution and Ca^{2+} concentration were chosen for this study and

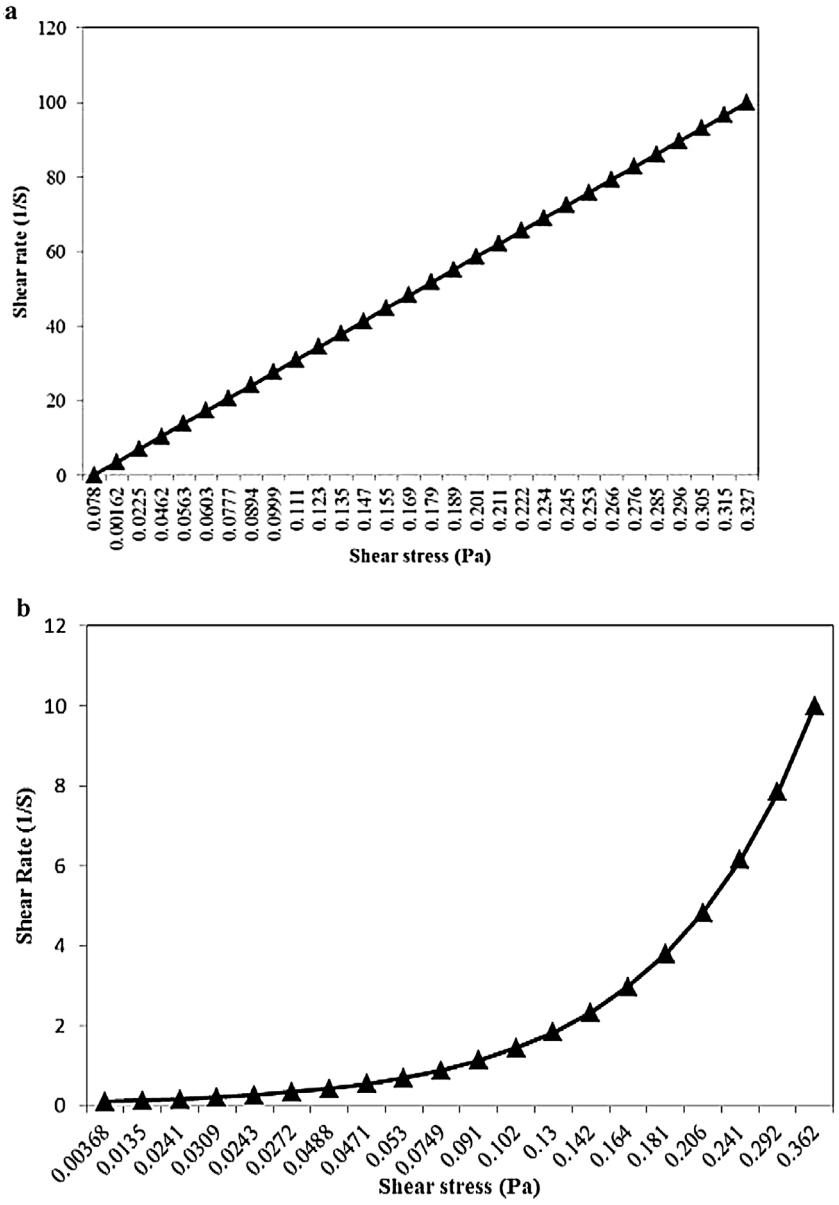

Fig. 4. Shear stress versus shear strain of biopolymer at concentrations of a) 2 g/L and b) 5 g/L.

given in Table 1. Since lipopeptides precipitate at acidic conditions (pH below 6), the level in the design was chosen such that the minimum experimental pH was above 6. A central composite design (CCD) was employed for three factors and the experimental design was obtained by using Design Expert version 7.0, which resulted in 20 different combinations of input variables with% oil recovery as response as shown in Table 2. The process parameters were varied on the basis of the CCD table and the amount of oil recovered was recorded.

2.6.2. Optimization by ANN coupled with GA

MATLAB version 8.0 (Mathworks Inc., Natick, USA) was employed to carry out ANN-GA optimization. An ANN model was developed and trained by using the CCD data with feed-forward back propagation (BP) algorithm. The network topology involves an input layer, a hidden layer and an output layer. The connections between each layer are generally expressed in terms of weights and bias. During training, the network starts with random weights and bias values and executes an activation function (Eq. (1)) which consequently produces a simulated output. The error% between experimental output and simulated output would be substantially reduced by iteratively feeding the error backwards using BP algorithm and updating the values of weights and bias. Subsequently, optimal network architecture was determined so as to eliminate over-fitting of data. The mean squared error (MSE) (Eq. (2)) and overall correlation coefficient (R) were used as the performance indices, in order to develop an optimal model.

$$Y_j = \sum (x_i w_{ij}) + b_j \tag{1}$$

where, Y_j = activation function, w_{ij} = weight connection between neurons i and j, x_i is the input at neuron i and b_j is the bias of neuron j.

$$MSE = \sum \frac{(Experimental\ value - predicted\ value)^2}{n} \tag{2}$$

Once the ANN model was developed to the desired level of precision, GA was used to determine the optimal combination of process parameters for achieving maximum oil recovery. GA modifies a set of individuals (population) repeatedly by producing off-springs from individuals in the current generation. The best individuals in the population at successive stages converge to the actual solution. The initial population of 40 individuals was created using a uniform

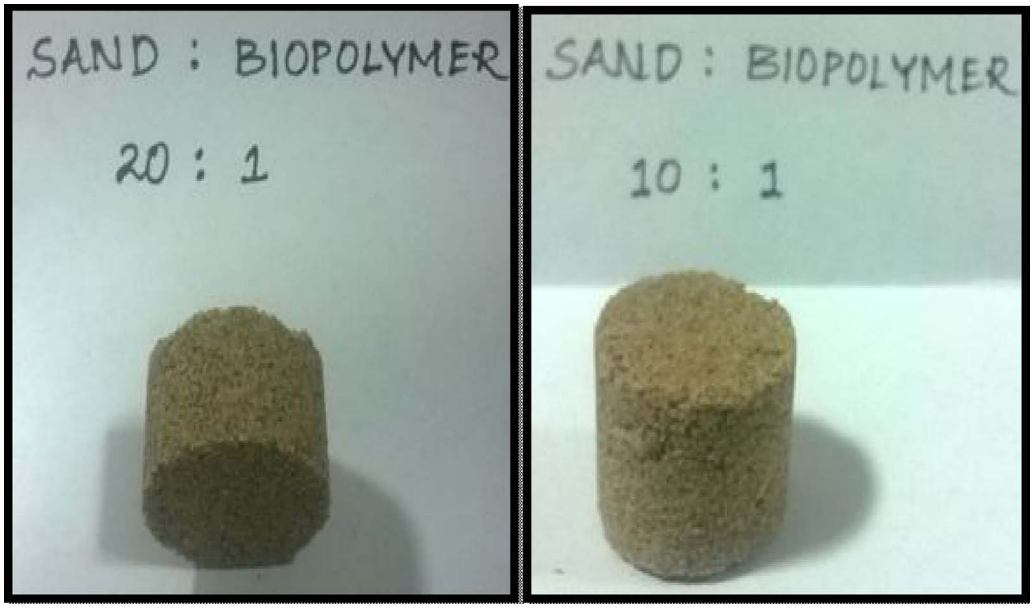

Fig. 5. Cylindrical sand blocks made using biopolymer and sand.

creation function from the given range. Thus each of the 40 individuals represented a chromosome comprising of 4 different genes; each gene, in turn, representing the concentration of a particular media component. Every individual in the population was scored according to its fitness value obtained using the ANN model. The initial population strategy is applied to reduce the number of searches to reach the optimum design in the solution space. After ranking all the members of the population according to their fitness, they were subjected to three main operations – selection, crossover and mutation. This sequence of fitness computation, selection and reproduction was carried out for 100 generations to ensure suitable convergence.

3. Results and discussion

3.1. Characterization of lipopeptide biosurfactant for enhanced oil recovery

Lipopeptide biosurfactants are widely reported for its application in cosmetics and food industry, pharmaceutical industry, agricultural industry and enhanced oil recovery, owing to its higher biodegradability, low toxicity and high surface activity (Rangarajan et al., 2015; Rangarajan and Clarke, 2016). The use of crude lipopeptide cocktail, i.e. the mixture of lipopeptides including surfactins, iturins and fengycins (Dhanarajan et al., 2016), obtained after acid precipitation of culture supernatant, as used in the current study, would prove to be economically viable, since the product is used without cost-intensive downstream processing procedures. Also, the use of cheaper substrate sources in the production of lipopeptides will improve the overall bioprocessing economy, thus making lipopeptide a potential candidate for enhanced oil recovery. Biosurfactants injected into the depleted oil wells essentially reduces the interfacial tension between the rock and oil, thereby help releasing the oil from the capillary interstitial spaces. Forming emulsion with the oil, the biosurfactant forms a stream of continuous single phase comprising oil and water, which can be easily mobilized by a suitable flooding solution. Firstly the two important characteristics of the crude lipopeptide cocktail such as emulsification activity and surface tension reducing ability were evaluated. Subsequently, the effect of Ca^{2+} ions on the surface tension of crude lipopeptide solutions was studied.

3.1.1. Emulsification activity

Emulsification activity of the lipopeptide biosurfactant produced by the marine *B. megaterium* was measured at various concentrations and as expected E_{24} index increased with the increase in concentration. It showed stable emulsification activity at above 250 mg mL^{-1} as shown in Table. 3. A maximum emulsification value of 81.66% was obtained at a lipopeptide concentration of 2.5 g L^{-1}, beyond which there was no significant improvement in emulsification activity. The E_{24} values obtained in this study were found to be comparable with that in previous literature reports. A lipopeptide biosurfactant synthesized by *Pseudomonas nitroreducens* showed emulsification activity of 40–60% for various hydrocarbons and oils (de Sousa and Bhosle, 2012), whereas that from *Bacillus subtilis* K1 exhibited in the range of 30–55% (Pathak and Keharia, 2014). Thus, the results indicated that the biosurfactant could be a potential agent in enhanced oil recovery for it showed stable emulsification activity with oil.

3.1.2. Surface and interfacial tension measurements

The CMC of crude lipopeptide biosurfactant in water at pH 8 was found to be 250 mg L^{-1} (Fig. 1a), giving a minimum surface tension of 28.25 mN/m. The low CMC obtained could be attributed to the presence of other fermentation impurities. The biosurfactant reduced the interfacial tension between water to oil from 18.6 to 1.5 mN/m (Fig. 1b).

3.1.3. Effect of Ca^{2+} addition to biosurfactant solution

Ca^{2+} ions were added to the biosurfactant solution as $CaCl_2.2H_2O$ in the concentration of 0.2–2 mM and the resulting surface tension reduction was measured as shown in Fig. 2. It could be observed that Ca^{2+} decreased the surface tension of crude lipopeptide solution from 37.4 mN/m to 26.9 mN/m at a Ca^{2+} concentration of 2 mM. Ca^{2+} ions was reported to have strong affinity towards carboxylate functional groups of peptide moities and thus stabilize the lipopeptide micelles by increasing the monomer packing density as a result of reduced CMC (Rangarajan and Sen 2013). Thus a Ca^{2+} conditioned lipopeptide solution is expected to yield to stable lipopeptide micellar solution that can result in improved oil recovery.

3.2. Characterization of biopolymer for MEOR application

Bacillus licheniformis was grown in GMSM at 60 °C in an incubated shaker at 150 rpm for 24 h. The product was found to be growth associated. A maximum product concentration of 6.2 ± 0.7 g L^{-1} was obtained after 24 h of growth.

Bacterially-derived biopolymers like xanthan has been widely used

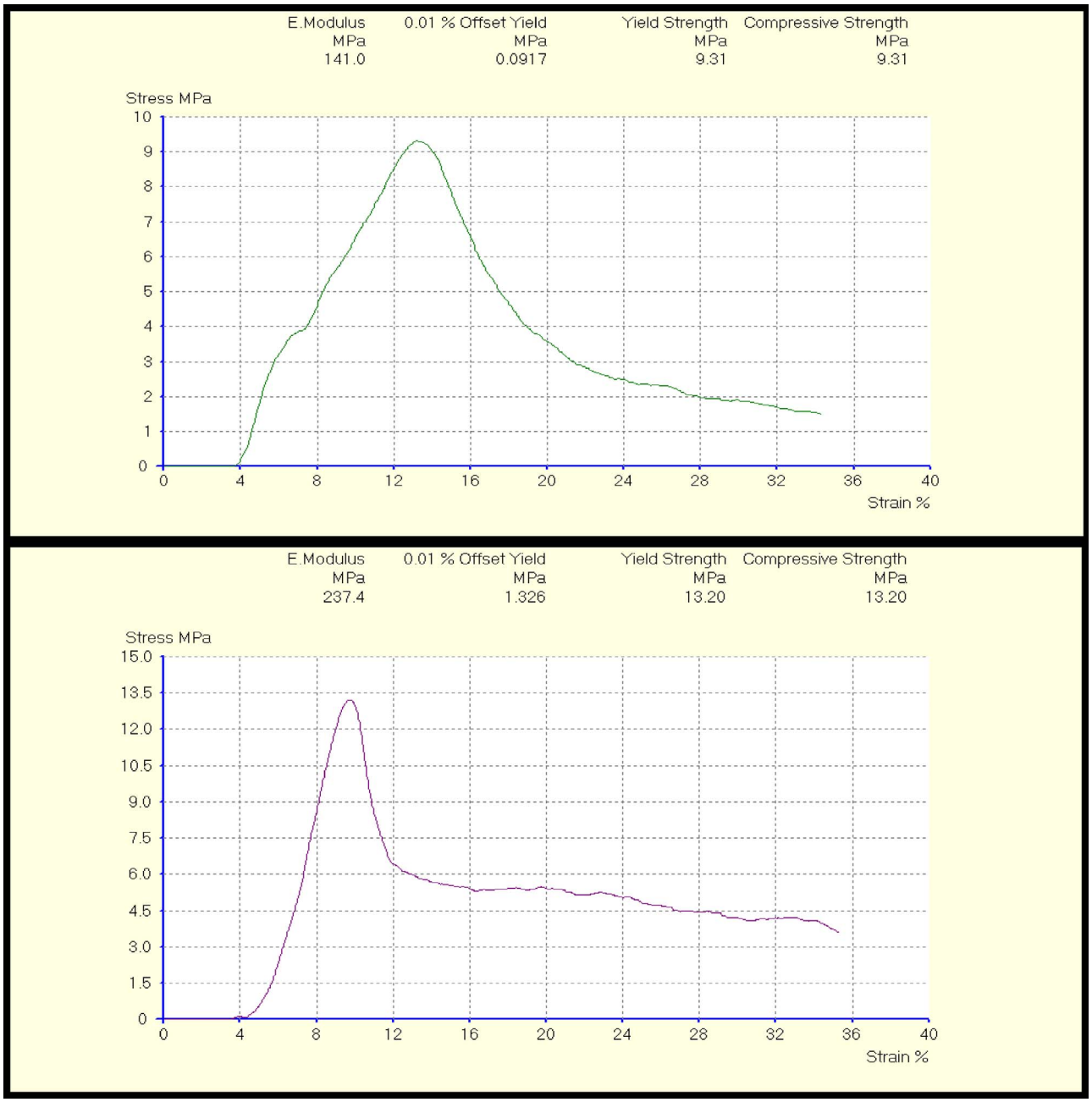

Fig. 6. Mechanical strength of sand-biopolymer biocomposite at a) 10:1 and b) 20:1 of sand-to-biopolymer ratio (w/w).

as viscosifying agent in flooding applications in MEOR (Patel et al., 2015). Recently, Gao (2016) tested another novel polymer namely Schizophyllan obtained from the fungus *Schizophyllum commune* for enhanced oil recovery. Another role of biopolymers is to selectively plug the thief-zones, which would otherwise significantly reduce sweeping efficiency of flooding agent in tertiary oil recovery operations. Another approach in MEOR is the direct injection of biopolymer-producing organism into the depleted wells, which can seal the pores, by bacterial adhesion facilitated by the biopolymers (Patel et al., 2015; Sen, 2008). With these potential applications of biopolymer in MEOR, it is very important to characterize some of the important characteristics of biopolymer such as viscosity, pore plugging ability and bio −cementing ability for MEOR applications.

3.2.1. FTIR analysis

Fig. 3 shows the FTIR spectrum of the biopolymer. Characteristic broad stretching of −OH group was observed at 3316.55 cm^{-1}, weak −C−H vibration was observed at 2936.35 cm^{-1}. The wavelengths between 817 cm^{-1} to 1400 cm^{-1} as shown in Table 4 are very important as they act as fingerprint for the identification of levan (Barone and Medynets 2007; Jathore et al., 2012). Thus, the initial characterization of the biopolymer showed that the biopolymer might belong to the levan family. Although the current study gives considerable insight about the characteristics of the product produced by *Bacillus licheniformis*, a comprehensive study with the pure product is required to elucidate the actual structure of the compound.

3.2.2. Rheological studies

Biopolymer solutions of different concentrations (1 g L^{-1}–5 g L^{-1}) were prepared in water. The solutions exhibited Newtonian's behaviour up to the biopolymer concentration of 2 g L^{-1}. Fig. 4a shows the effect of shear stress on the shear rate of the solution (2 g L^{-1}), a constant viscosity of 3.5 cP was observed. However, at high biopolymer concentrations, the shear rate was not proportional to the applied shear

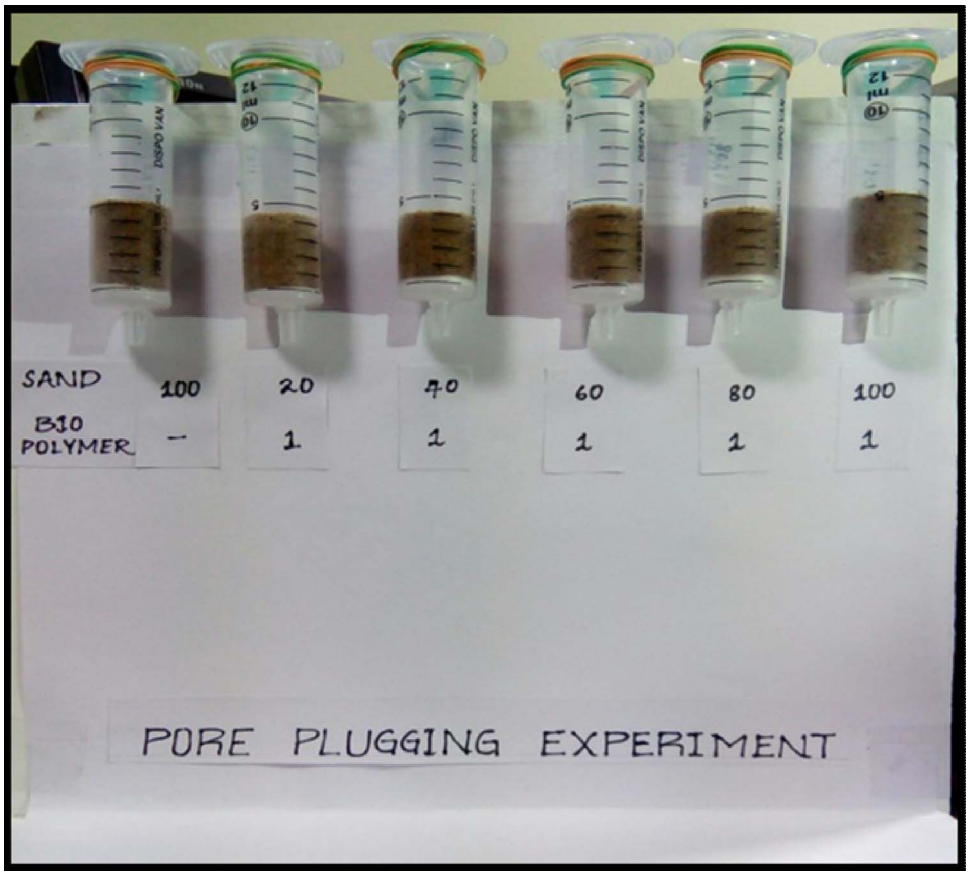

Fig. 7. The experimental set-up for pore plugging studies (Each column contained uniform amount of 7.2 g of sand).

Table 5
Wettability of sand-biopolymer packing.

Mass ratio (sand/biopolymer) (g/g)	Time to reach 50% packing height (min)	Time to reach 100% packing height (min)
20:1	80 ± 3.36	101 ± 0.4
40:1	4.65 ± 0.22	6.0 ± 0.25
60:1	0.47 ± 0.02	1.5 ± 0.06
80:1	0.35 ± 0.01	1.1 ± 0.05
100:1	0.33 ± 0.01	0.8 ± 0.04
Control (only sand)	0.2 ± 0.01	0.4 ± 0.02

stress or the viscosity of the solution varied with the applied shear stress (Fig. 4b), indicating non-Newtonian behaviour of the solution. Furthermore, the rheology of solutions showed a pseudo plastic behaviour at biopolymer concentrations greater than 2 g L^{-1} (Fig. 4b). At a biopolymer concentration of 5 g L^{-1} the apparent viscosity of the solution for applied shear stress of 0.0243 Pa was found to be 90 cP. The rheological studies of the biopolymer solutions were found to be promising and further suggested that the biopolymer could serve as a potential viscosifying agent for flooding application in MEOR.

3.2.3. *Bio-cementing and bio-plugging studies*

The cylindrical solid sand blocks (Fig. 5) with flat surfaces prepared

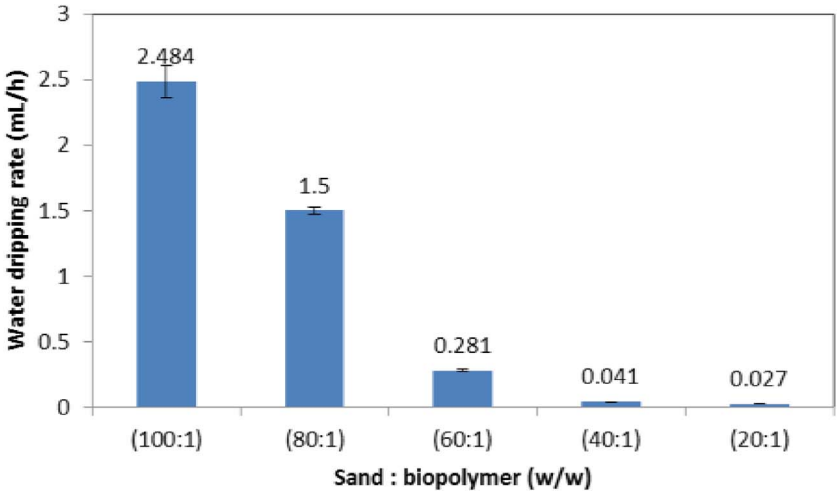

Fig. 8. Water dripping rate in sand pack columns at various sand to biopolymer ratio (w/w).

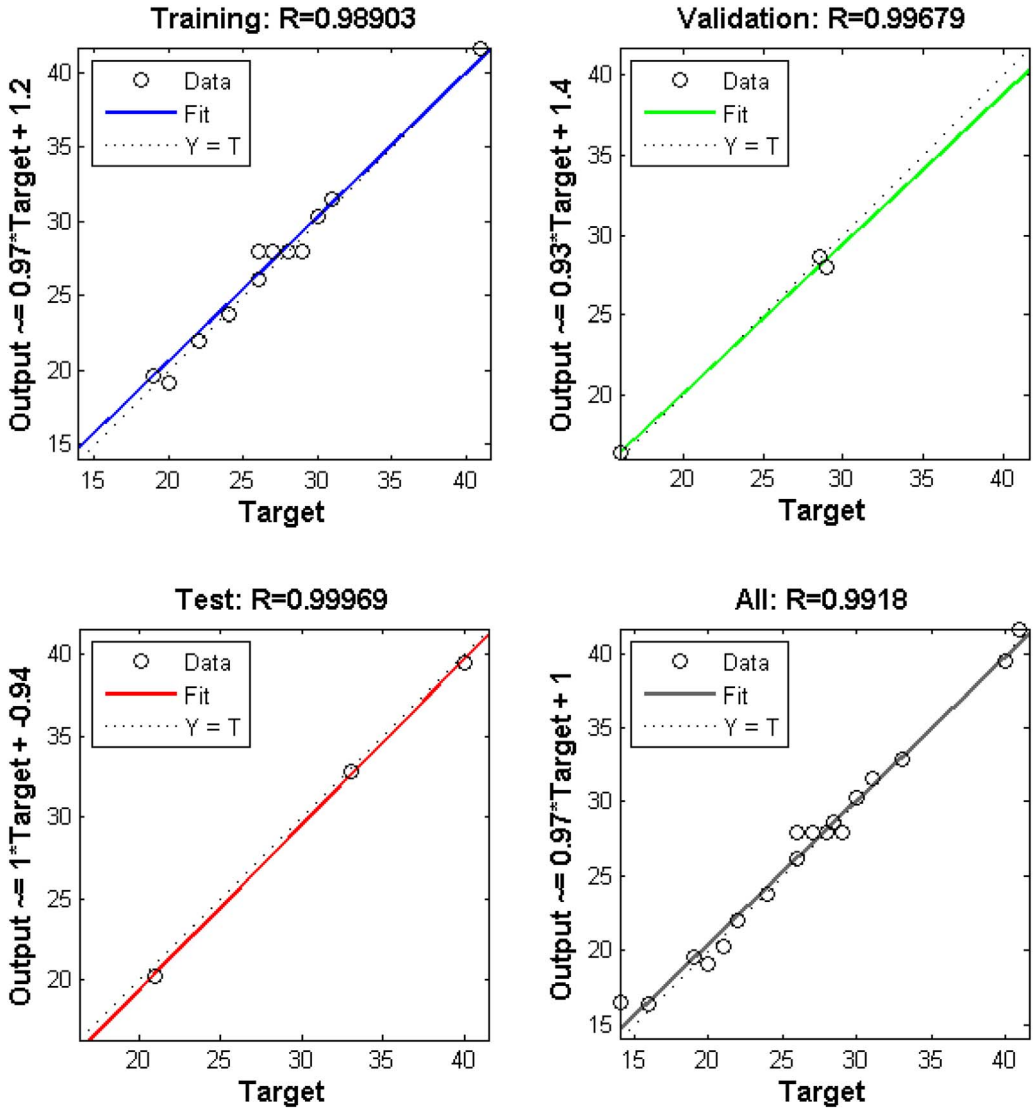

Fig. 9. Regression plot of predicted and experimental outputs.

using biopolymer as described before was subjected to compression studies. Maximum compression strengths of 13.2 ± 0.4 M Pa and 9.31 ± 0.7 M Pa and elastic modulus of 237.4 ± 2.4 M Pa and 141 ± 1.8 M Pa were recorded for the solid-sand block with 10:1 (Fig. 6a) and 20:1 (Fig. 6b) (sand: biopolymer% w/w) respectively.

Pore plugging ability of the biopolymer was demonstrated by testing the water arresting ability of biopolymer in the sand pack-column. The experimental set up for demonstrating bio-plugging ability is shown in Fig. 7. The sand-packed columns with different amount of biopolymers showed that product was able to plug the pores uniformly over the sand packed column. The percolation of water into the sand-only columns (used as control) was so rapid that water dripped from bottom opening after 24 s addition over the sand layer. On the other hand, water placed over the biopolymer-sand packed column, was completely impervious to the flow of water even after a day. The initial wettability studies with 20:1 w/w of sand: biopolymer showed that water could diffuse and wet half of the depth of the bed only after 80 min and it took nearly 101 min to completely wet the sand bed. Also, it was observed that the column was so compact that it did not show any swelling even after 24 h. However, it took only 6 min to completely wet the column at Sand: biopolymer ratio of 40:1. The wettability characteristics of sand packed columns at different ratio of sand: biopolymer is shown in Table 5. Once the water completely wetted the column, the gravity caused water to drip through the column. Fig. 8 shows water dripping rate at different sand: biopolymer ratio. It could be seen that only 0.027 mL of water was collected in 20:1 ratio after an hour, which showed that biopolymer plugged the sand pores very effectively that it almost completely prevented the flow of water. The dripping rate was almost constant for a period of two days. However, as the sand: biopolymer ratio was increased from 20:1 to 100: 1 the dripping rate increased from 0.027 mL h^{-1} to 2.494 mL h^{-1}. One of the proposed mechanisms for pore plugging ability could be due to the swelling ability of biopolymer in the presence of water. The resulting water-trapped biopolymer acted as gel and completely blocked the sand pores. Thus the observed decrease in pore plugging ability with the decrease in the biopolymer amount might be attributed to the reduction in size of biopolymer gel network that plug the void spaces of the sand column. Under this hypothesis, it could be predicted that water could only diffuse through the biopolymer gel, which caused only a meagre dripping of water at a low sand:biopolymer ratio of 20:1 (w/w).

The results of viscosity, biocementing and bioplugging studies indicate that the crude biopolymer obtained from thermophilic *Bacillus licheniformis* can become a potential candidate for application in MEOR. They can essentially serve as viscosifying agent in flooding applications and also as plugging agent to seal the thief zones which can result in the enhanced oil recovery. Further, it is also strongly believed that the thermophilic organism *B. licheniformis* itself can be used for insitu MEOR applications.

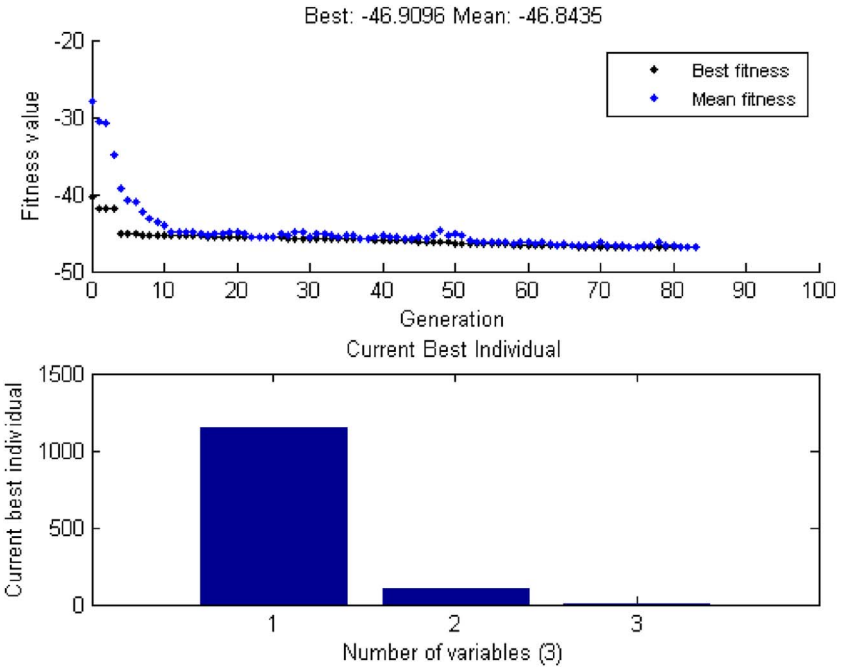

Fig. 10. Fitness plot, obtained after optimization by GA. Best and average fitness values show a gradual convergence towards the optimum value after 83 generations.

3.3. ANN-GA optimization of process variables for enhanced oil recovery in a sand – packed column

The efficiency of biosurfactant and biopolymer to recover oil was carried out in a lab-scale sand-packed column, saturated with lube oil. The injection fluid contained lipopeptide biosurfactant at varying concentration supplemented with Ca^{2+} ions and whose pH was adjusted according to the CCD design as shown in Table 2. A biopolymer solution of 3 g L^{-1} was used as a flooding solution in all studies.

As mentioned in the methodology, three important parameters were chosen for optimization studies in sand pack column. In order to develop a suitable ANN model, CCD experimental data (Table 2) was appropriately segmented as follows: 70% for training, 15% for testing and 15% for validating. The network trains by utilizing maximum data points and it also performs self-tests and validates at each run, with a view to avoid over-fitting of data. The overall performance of the network depends on the type of transfer functions used to train the neural network (Huang et al., 2007). It was observed in this study that tangent-sigmoidal transfer function at the hidden layer node and pure-linear transfer function at the output layer node were found to be more efficient, in terms of minimum MSE and maximum R-values. As the number of neurons in the hidden layer critically influences optimal network design, the optimal number of hidden neurons was determined as described in our previous study (Dhanarajan et al., 2014). A minimum MSE value of 0.403 and maximum R-value of 0.9918 (Fig. 9) was obtained for 12 neurons in hidden layer. As the correlation co-efficient R-value is close to 1, it indicates that the developed ANN model did not over-fit the data obtained between the simulated and experimental responses. Hence, 2-12-1 network topology was chosen and the model was used as fitness function in GA tool for finding suitable combination of process parameters for enhanced oil recovery.

Upon running the GA, the maximum oil recovery was predicted for the optimal process conditions as follows: lipopeptide concentration, 1110 mg L^{-1}; Ca^{2+} concentration, 74 mg L^{-1} and pH, 8.3. The fitness plot (Fig. 10) obtained from GA gradually converged towards the final optimum (46.8% oil recovery), after 83 generations. It was validated by running the GA several times using different randomly selected initial populations. The results of five successive runs showed very slight variation in the values of process output values as well as oil recovery%. Since several such runs of the code yielded very consistent and similar results, it was concluded that the GA was able to determine the global maxima with high accuracy.

3.4. Model validation studies

Experimental validation studies were conducted in triplicates in the sand packed column using the optimized process conditions. The maximum oil recovery obtained from the optimized process conditions was compared with the model predicted values. The error% between the experimental (45%) and model predicted (46.8%) values were found to be approximately 4%, which is in the acceptable range. This indicated the efficiency of the ANN-GA technique to optimize the process variables for enhanced oil recovery.

4. Conclusion

The flooding strategies currently adopted for enhanced oil recovery need to be intensified in order to effectively recover the untouched two-thirds of crude present in the pre-explored oil reservoirs. Production and testing of microbially-derived products such as biosurfactants and biopolymers with improved emulsifying abilities and oil-sweeping efficiencies are the need of the hour towards further improving the oil recovery. In the current study, we demonstrated the application potential of biosurfactant and biopolymer as potential alternatives to their synthetic counterparts for the enhanced oil recovery using a sand-packed column. As an effort to improve the stability of the biosurfactant solution, the lipopeptide solution was conditioned using Ca^{2+} ions. The Ca^{2+} conditioned lipopeptide in its crude form was proved to be effective at a concentration as low as 1.1 g L^{-1}. A maximum oil recovery of 45% could be achieved using a flooding solution, viscosified using bacterially-derived- biopolymer at a concentration of 3 g L^{-1}. Based on these preliminary studies, it is recommended that the future studies should be aimed at investigating the combined properties of both biosurfactant and biopolymer in the injection solution and the resulting effect in the enhanced oil recovery.

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